AR 201-12930A

The Flavor and Fragrance High Production Volume Consortia

The Terpene Consortium

Test Plan for Terpenoid Tertiary Alcohols and Related Esters

Linalool (3,7-dimethyl-1,6-octadien-3-ol)	CAS No. 78-70-6
Tetrahydrolinalool (3,7-dimethyl-3-octanol)	CAS No. 78-69-3
Myrcenol (2-methyl-6-methylene-7-octen-2-ol)	CAS No. 543-39-5
Dihydromyrcenol (3,7-dimethyl-7-octen-2-ol)	CAS No. 18497-58-8
Linalyl acetate (3,7-dimethyl-1,6-octadien-3-yl acetate	CAS No. 115-95-7
alpha-Terpineol (*p*-menth-1-en-8-ol)	CAS No. 98-55-5
alpha-Terpinyl acetate (*p*-menth-1-en-8-yl acetate)	CAS No. 80-26-2
2,6,6-Trimethylbicyclo[3.1.1]heptan-2-ol thermal rearrangement products, linalool fractions, acid-isomerized, distn. residues, acid-isomerized, distn. lights, terpenoids	CAS No. 125252-49-5
cis-2-Pinanol	CAS No. 4948-28-1
2-Pinanol	CAS No. 473-54-l
trans-2-Pinanol	CAS No. 4948-29-2
2-Pinanol hydroperoxide	CAS No. 28324-52-9
Pine oil	CAS No. 8002-09-3

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The Flavor and Fragrance High Production Volume Chemical Consortia

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List of Member Companies

Sponsoring Substances in Tertiary Terpenoid Alcohols and Related Ester Derivatives

Arizona Chemical

BASF

Bush Boake Allen, Inc.

Citrus and Allies Essences, LTD

Givaudan Roure

ICI Americas

International Flavors and Fragrances Inc.

J. Manheimer Incorporated

Hercules Chemical

Millennium Chemicals, Inc.

TECNAL Corporation

Universal Flavor Corporation

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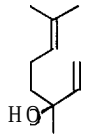
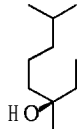
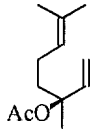
1 Identity of Substances



3,7-Dimethyl-1,6-octadien-3-ol
Linalool
$C_{10}H_{18}O$
CAS No. 78-70-6



3,7-Dimethyloctan-3-ol
Tetrahydrolinalool
$C_{10}H_{22}O$
CAS No. 78-69-3



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Linalyl acetate
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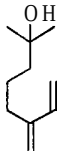

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Myrcenol
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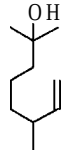

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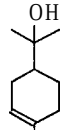

p-Menth-1-en-8-ol
alpha-Terpineol
$C_{10}H_{18}O$
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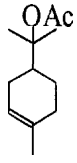

p-Menth-1-en-8-yl acetate
alpha-Terpineol acetate
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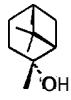
CAS No. 80-26-2

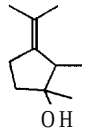
cis-2-Pinanol
$C_{10}H_{18}O$
CAS No. 4948-28-1



trans-2-Pinanol
$C_{10}H_{18}O$
CAS No. 4948-29-2

2-Pinanol
$C_{10}H_{18}O$
CAS No. 473-54-1

2-Pinanol hydroperoxide
Pinane hydroperoxide
$C_{10}H_{18}O_2$
CAS No. 28324-52-9



2,6,6-Trimethylbicyclo [3. 1.1]heptan-2-ol thermal rearrangement products
CAS No. 125252-49-5
Major isomer: plinol
$C_{10}H_{18}O$
CAS No. 72402-00-7

2 Category Analysis

2.1 Introduction

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Terpene Consortium, as a member of FFHPVC serves as an industry consortium to coordinate testing activities for terpenoid substances under the Chemical Right-to-Know Program. Seventeen (17) companies are current members of The Terpene Consortium, twelve (12) of which are sponsoring substances in this chemical category. The Terpene Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing. The category analysis, test plan, and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

2.2 Background Information

In nature terpenes are produced by the isoprene pathway, which is an integral part of normal plant and animal biosynthesis. They are ubiquitous in the environment and in food consumed by humans and other animals. Linalool and alpha-terpineol are two of the most common terpenoid alcohols, occurring to varying degrees in essentially all plants [Lawrence, 1985]. Linalool accounts for more ***than 70%*** of ***the*** spice essential oil, coriander oil. A mixture of *alpha*-terpineol and its acetate ester, linalool and its acetate ester, and other tertiary terpenoid alcohols account for more than 70% of cardamom oil [Gopalaluishnan and Narayan, 1991]. Linalool and ***alpha-terpineol, the*** two parent alcohols in this category, are widely dispersed in fruits and vegetables as well as the fermented products prepared from these foods. Principal exposure to

these two terpenes arises via the consumption of spices, carrots, orange juice, nutmeg, beer, wine, and tea [Stofberg and Grundschober, 1987]. Ten (10) of the 13 substances in this chemical category are currently recognized by the US. Food and Drug Administration (FDA) as GRAS ("generally regarded as safe") for their intended use as flavoring substances [Hall and Oser, 1965]. However, quantitative data on natural occurrence indicate that oral intake of these substances occurs predominantly from consumption of food in which they occur naturally [Stofberg and Grundschober, 1987; Stofberg and Kirschrnan, 1985]. For example, greater than 200,000 pounds (lbs) of linalool and alpha- terpineol, and their corresponding acetate esters are consumed annually as natural components of traditional foods not including spices [Stofberg and Grundschober, 1987]. Including the consumption of spice and spice oils, intake of these alcohols exceeds 1,000,000 lbs annually [Lawrence, 1985]. On the other hand, the intentional use of these materials in flavors results in the annual consumption of less than 50,000 lbs [Lucas ***et al.,*** 1999].

2.3 Structural Classification

The chemical category designated "Tertiary Terpenoid Alcohols and Related Esters" includes eight terpenoid aliphatic tertiary alcohols and two related acetate esters. The group of alcohols consist of linalool (3,7-dimethyl- 1,6-octadien-3-ol), tetrahydrolinalool (3,7-dimethyl-3-octanol), a tertiary alcohol isomer of linalool, myrcenol (2-methyl-6-methylene-7-octen-2-ol), dihydromyrcenol (3,7-dimethyl-7-octen-2-ol); ***an*** isomeric alicyclic tertiary alcohol *alpha*-terpineol (*p*-menth- 1 -en-8-ol), 2-pinanol, and ***cis***- and *trans*-2-pinanol. The two esters include the acetate ester of linalool (3,7-dimethyl- 1,6-octadien-3-yl acetate) and acetate ester of ***alpha-terpineol*** (*p*-menth- 1-en-8-yl acetate).

This chemical category also includes a site-restricted peroxide of a terpene tertiary alcohol (pinane hydroperoxide) that is efficiently reduced to the corresponding alcohol (2-pinanol). It also includes two mixtures consisting predominantly of tertiary terpenoid alcohols. These two mixtures are pine oil and "2,6,6-trimethylbicyclo[3.1.1]heptan-2-ol thermal rearrangement

products, linalool h-actions, acid-isomerized distillation residues, acid-isomer&d distillation lights, terpenoids". The composition of these mixtures is discussed below.

Seven of the ternary alcohols are isomers of the formula $C_{10}H_{18}O$, one is a dihydro homologue ($C_{10}H_{20}O$), and one is a tetrahydro homologue ($C_{10}H_{22}O$). The two acetate esters have the formula $C_{12}H_{20}O_2$. The pinane hydroperoxide has the formula $C_{10}H_{18}O_2$. The two mixtures are composed principally of terpenoid tertiary alcohols of the formula $C_{10}H_{18}O$.

2.3.1 Pinane hydroperoxide

Pinane hydroperoxide is a mixture of approximately 75% *cis*- and 25% *trans*-isomers [Canova, 1977]. Commercially it is a site-restricted chemical intermediate, which is formed by the oxidation of ***alpha-pinene*** with molecular oxygen. The vast majority of industrial pinane hydroperoxide is reduced with sodium bisulfite or other reducing agents to yield mixtures of the commercially important alcohols, *cis*-2-pinanol (55-56%) and *trans*-2-pinanol (25-27%). Small amounts of terpene hydrocarbons (limonene, ***alpha***- and beta-pinene, menthene) and other terpenoid alcohols ***(alpha-terpineol,*** isopinocampheol, isoverbanol, bomeol) are also formed [Gorman, 1976; Brose et ***al.,*** 1992]. ***Cis***- and *trans*-2-pinanol are then separated and thermally converted to (+)-linalool and (-)-linalool, respectively.

Pinane hydroperoxide is a relatively stable peroxide with a shelf half-life greater than 100 hours at 120°C. During transportation it is recommended that the peroxide be stored in tightly closed containers at temperatures less than 35°C [Millennium Specialty Chemicals, 1999]. In addition to being a source of *cis*-2-pinanol and *trans*-2-pinanol, pinane hydroperoxide acts as a polymerization initiator in the production of vinyl, nitrile, styrene-butadiene, and polyester resins [Millennium Specialty Chemicals, 1999]. Similar to other tertiary aliphatic peroxides, pinane hydroperoxide [Milas and Surgenor, 1946] undergoes decomposition to yield 2-pinanol and other terpenoid alcohols. To a minor extent, it rearranges via alpha-cleavage to form ketones [Schmidt and Fisher, 1959].

2.3.2 Pine oil

Although a minor amount of commercial pine oil is still derived from natural sources (i.e., pine trees), the vast majority of pine oil in use today is synthetic in nature. Originally, pine oil was obtained by the steam distillation of crude turpentine from pine trees, mainly stumps rich in terpene oils. Today, it is produced predominantly by acid-catalyzed hydration of pinene. The definition of pine oil reflects its isolation from natural sources. According to the Toxic Substances Control Act [TSCA-PL 94-469, Addendum III, Chemical Substances of Unknown or Variable Composition, 1978] pine oil is defined as "a complex combination of terpenes produced by high temperature distillation of oil of turpentine residues or by catalytic hydration of pinene. It is composed primarily of tertiary and secondary terpene alcohols."

The composition of pine oil obtained by steam distillation varies depending on pine tree feed stocks and processing. The major constituent, alpha-terpineol (8-terpineol) accounts for 50-60% of the oil by weight. Other tertiary terpenoid alcohols, such as 1- and 4-terpineol, account for 15-25% of the oil with the remainder being composed of terpene hydrocarbons (5- 10%), secondary terpenoid alcohols, mainly fenchol and bomeol (5-10% each), terpene ethers (5-10%), ketones, and phenols (1-2%). High-grade natural pine oil may contain as much as 70% alpha-terpineol [Guenther, 1952].

The composition of synthetic pine oil derived from hydration of pinene is also predominantly composed of tertiary terpenoid alcohols. Specifically, the composition of pine oil used in toxicologic studies performed from 1990- 1991 was a blend of commercial oils from six major pine oil producers [Davis, 2000]. Multiple batch independent analysis [Toxikon, Inc., 1990] of this blend was found to contain 56.2% alpha-terpineol, 9.2% gamma-terpineol, 4.3% other terpineols (beta-terpineol, 4-terpineol), 6.5% secondary terpenoid alcohols (fenchol and bomeol), 20.4% terpene hydrocarbons (mainly limonene, p-menthadiene, and terpinolene), and <4% of aromatic and alicyclic terpene hydrocarbons and ethers. In addition to alpha-terpineol, some grades of commercial pine oil may also contain minor amount of *cis* and *trans*-2-pinanol, alpha-terpineol acetate, and linalool [Millennium Specialty Chemicals, 2000]. The pine oil blend

used in toxicity studies performed in 1994 consisted of 62.7% alpha-terpineol and 83.4% total terpene alcohols [Villa, 1994]. Pine oil was blended from five commercially available lots of pine oil and subjected to toxicity studies from 1985-1987. The range of concentrations of terpene constituents in the five lots contained 35- 76.7% ***alpha***- and gamma-terpineol, 1- 13.1% other terpineol isomers, 0- 14.6% secondary terpene alcohols, 0. 1- 10% terpene hydrocarbons [Hercules, 200 1].

Prior to 1970, toxicology studies were performed on commercial grades of pine oil commonly recognized as Yarmor. Similar to commercial grades of pine oil available today, Yarmor contains 50-60% alpha-terpineol in combination with other terpineols (10- 15%), linalool (1%), secondary terpenoid alcohols (7-15%), and terpene hydrocarbons (10-20%) [CSMA, 2000]. Based on these data, it can be concluded that commercial pine oil and pine oil subjected to toxicity studies is composed of 60-80% terpineol isomers, ***mainly the alpha*** isomer. The remaining constituents are primarily secondary terpenoid alcohols and terpene hydrocarbons.

2.3.3 2,6,6-Trimethylbicyclo[3.1 .1]heptan-2-ol - thermal rearrangement products

The rearrangement products from thermolysis of pinanol (2,6,6-trimethylbicyclo [3.1. 1]heptan-2-ol) include plinol (1,2-dimethyl-3-isopropenylcyclopentanol, 47-50%), *trans*-2-pinanol (2 1-24%), linalool (7-9%), *cis-beta*-terpineol (1-2%), *p*-menthan- 1-ol (1-2%) and the secondary bicyclic alcohol alpha-fenchol (<4%). The remaining components each of which accounts for less than 1% of the mixture are simple aliphatic alcohols and terpenoid hydrocarbons (geraniol, myrcene, limonene, and alpha-pinene) [Millennium Specialty Chemicals, 2000]. The major isomer plinol (CAS No. 72402-00-7) is a tertiary alcohol and is structurally related to *alpha*-terpineol. Because of its ready hydrolysis to plinol, plinyl acetate (1,2-dimethyl-3-isopropenylcyclopentanyl acetate) is considered a good closely related structural model for this product.

The thirteen substances are assigned to the same chemical category because of their close structural relationships, their similar physio-chemical properties, including the ready conversion of linalool to alpha-terpineol. They are also considered together by virtue of the fact that they

participate in the same pathways of metabolic detoxication and have similar toxicologic potential.

2.4 Industrial and Biogenic Production

2.4.1 Industrial Production

(+/-)-Linalool and its esters are the most frequently used in fragranced products. However, most of the manufactured linalool is used in the preparation of Vitamin E. Like its enantiomers, the racemic form (+/-)-linalool provides a flowery-fresh fragrance, reminiscent of lily of the valley. Although it can be isolated from essential oils such as Brazilian rosewood oil, most linalool used in fragrances and flavors is produced synthetically. Preparation from alpha-pinene via pinane hydroperoxide selectively yields the optically active (+) and (-) forms of linalool [Canova, 1977]. An alternate synthesis involves sequential conversion of beta-pinene to myrcene, which in turn, is converted to linalyl chloride in the presence of HCl and copper (I) catalysts. The chloride is then converted to linalyl acetate, which may be used as is or saponified to linalool [Webb, 1964].

Myrcenol is prepared from myrcene by addition of HCl, followed by hydrolysis [Blumenthal, 1966]. Likewise, dihydromyrcenol is formed from hydrohalogenation and hydrolysis of 2,6-dimethyl-2,7-octadiene, a thermal degradation product of *cis*-pinane [Webb, 1960]. Tetrahydrolinalool is prepared by the catalytic hydrogenation of linalool.

alpha-Terpineol is a highly important commercial product. Only small quantities are isolated from essential oils. An efficient synthesis involves the simple hydration of pinene from turpentine to yield *cis*-terpin hydrate followed by dehydration to yield alpha-terpineol [Hoyer et *al.*, 1959]. Selective hydration of pinene, delta-3-carene, and limonene or diterpene is also used to generate alpha-terpineol. The tertiary terpenoid alcohols *cis*- and *trans*-2-pinanol are synthesized from pinane via the hydroperoxide.

Commercially, the use of pine oil exceeds the annual use of all other members of this chemical category. Annual industrial production of pine oil is approximately 30,000,000-35,000,000 lbs of which only 4,000,000 to 5,000,000 lbs is derived from distillation of naturally occurring sources, mainly crude sulfate turpentine. The remainder is a synthesized product using pinene as a source material [PSMA, 2000]. Production of pine oil has changed significantly during the last half decade. Whereas synthetic pine predominates in the current marketplace, essentially all of the roughly 8,000,000 lbs of pine oil produced in 1950- 195 1 came from the distillation of crude turpentine [Goldblatt, 195 1].

2.4.2 Biogenic Production

Linalool, myrcenol, alpha-terpineol, and their related esters, homologues, and mixtures are common plant monoterpenoids that are ubiquitous in the environment. Most if not all vegetation produces these alcohols and esters. Environmental monitoring has detected ambient atmospheric [Larsen *et al.*, 1997] and aquatic [Heil and Lindsay, 1990] levels of tertiary terpenoid alcohols, The environmental impact of these substances must consider not only industrial sources and emissions, but also biogenic sources and emissions. Arguably, if background biogenic production and subsequent emission of terpenoid alcohols exceeds industrial (anthropogenic) production and emission by orders of magnitude, no significant environmental impact from industrial use and production can be expected.

Linalool, alpha-terpineol, myrcenol, and their corresponding acetate esters are present in almost every conceivable plant. Therefore, daily consumption of fresh fruit and vegetables and processed foods and drinks derived from these food groups provides exposure to these substances. Estimates of consumption of these substances as the result of food consumption exceeds 200,000 lbs annually [Stofberg and Grundschober, 1987]. This value represents a minute fraction of the biogenic production of these substances in the environment. For instance, consider the biogenic stores and biogenic production of tertiary terpenoid alcohols from pine trees, Based on the known relative amount of pine oil (tertiary terpenoid alcohols, mainly *alpha*-terpineol) in steam distilled wood turpentine obtained from longleaf and slash pine in the Eastern

U.S.A, it is possible to estimate the amount of these tertiary alcohols released from pine sources. One ton of air seasoned dry "fat" wood yields approximately 4 gallons or 14 lbs of pine oil, which is made up of approximately 10 lbs of tertiary terpenoid alcohols [Guenther, 1952]. One acre produces approximately 5 tons of viable pine stumps, the part of the tree richest in sources of turpentine and pine oil. Therefore approximately 50 lbs of tertiary alcohols are available per acre or 1,000,000,000 lbs for 20,000,000 acres of pine-forested land in the U.S.A. Although this simplified estimate is both crude and limited, it provides a perspective on the potential sources of tertiary terpenoid alcohols available from the environment.

2.5 Hydrolysis in Aqueous Media

The two terpenoid esters in this chemical category can be hydrolyzed to their corresponding terpenoid alcohol and acetic acid. Hydrolysis can be catalyzed by classes of enzymes recognized as carboxylesterases or Btype esterases [Heymann, 1980] that predominate in hepatocytes [Heymann, 1980]. Terpenoid alcohols formed in the gastrointestinal tract are rapidly absorbed [Phillips et *al.*, 1976; Diliberto *et al.*, 1988]. During hydrolysis under acidic conditions linalool may cyclize to form *alpha-terpineol* [Buck and Renwick, 1998; Buck and Renwick, 1999].

The esters of linalyl acetate and alpha-terpineol acetate are hydrolyzed under various *in vitro* and *in vivo* conditions *to* yield linalool or *alpha-terpineol*, respectively, and acetic acid. In an *in vitro* hydrolysis study, linalyl acetate was easily hydrolyzed in water, simulated gastric juice (pH 1.8), and pancreatic fluids (pH 7.5) [Buck and Renwick, 1999]. The mean half-lives for linalyl acetate hydrolysis in gastric juice with and without added hydrolytic enzymes was less than 2.5 minutes, respectively. Mean half-lives were less than 100 minutes in intestinal fluids in the absence or presence of pancreatin [Buck and Renwick, 1999]. Linalyl acetate also hydrolyzed in homogenates of rat intestinal mucosa, blood, and liver, but at rates much slower than in acidic gastric juice (rate constant for hydrolysis k=0.01 to 0.0055 min^{-1} versus greater than 5 min^{-1} in gastric juice). alpha-Terpineol acetate is also rapidly hydrolyzed in simulated gastric juice. The mean half-life in gastric juice is reported to be 16.4 minutes. In intestinal fluid (pH 7.5)

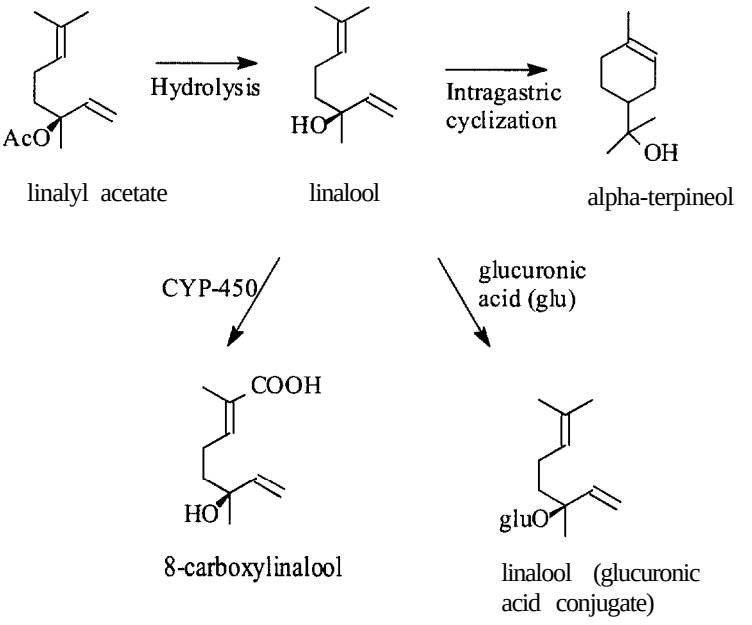
hydrolysis is somewhat slower (mean half-live, less than 400 minutes) [Buck and Renwick, 1999].

Carboxylesterase (Type B) activity also has been reported in a variety of fish species at different life stages [Leinweber, 1987; Boone et al., 1996; Abas and Hayton, 1997; Barr-on et ***al.,*** 1999]. Enzyme activity of rainbow trout sera, liver and whole body homogenates were similar to those of rat liver homogenate. A significant increase (300%) in activity occurred between yolk-sac and juvenile stage of rainbow trout development. Carboxylesterase activity was not significantly different for whole body homogenates of the rainbow trout, channel catfish, fathead minnows, and bluegill [Barron et ***al.,*** 1999]. These data support the conclusion that simple terpenoid esters, including linalyl acetate and alpha-terpineol acetate, are readily hydrolyzed in fish.

2.6 Reactivity in Aqueous Media

Following hydrolysis of linalyl acetate, resulting linalool partially undergoes ring closure to yield mainly alpha-terpineol and minor amounts of the terpenoid primary alcohols, geraniol and nerol (see Figure 1). In acidic (pH 1.8) artificial gastric juice, linalyl acetate is rapidly hydrolyzed ($t_{1/2}$<2.5 min.) to yield linalool. In neutral media (pH 7.5), hydrolysis is slower ($t_{1/2}$= <100 min.). Under both conditions of pH, approximately one-third of the resulting linalool is then rapidly rearranged to yield alpha-terpineol and small amounts of geraniol and nerol [Buck and Renwick, 1998; Buck and Renwick, 1999]. Based on these observations it can be concluded that linalyl acetate hydrolyzes under acidic conditions (i.e., gastric juice) to yield linalool which, to some extent, undergoes cyclization to yield ***alpha-terpineol.*** Both linalool and *alpha*-terpineol may then be either conjugated and excreted or oxidized to more polar excretable metabolites (see discussion below).

Figure 1. Metabolism of Linalool in Mammals



2.7 Metabolism of Tertiary Terpenoid Alcohols

In humans and animals, terpenoid tertiary alcohols primarily conjugate with glucuronic acid and are excreted in the urine and feces [Homing *et* al., 1976; Ventura *et* al., 1985; Parke *et* al., 1974a, 1974b; Williams, 1959]. Terpenoid alcohols with unsaturation may also undergo allylic oxidation to form polar diol metabolites that may be excreted either free or conjugated. If the diol contains a primary alcohol function, it may undergo further oxidation to the corresponding carboxylic acid [Madyastha and Srivatsan, 1988; Homing *et al.,* 1976; Ventura *et al.*, 1985].

The metabolic fate of the aliphatic tertiary alcohol linalool has been studied in mammals (see Figure 1). In rat liver homogenate, linalool or its CYP-450 oxidized metabolite is rapidly conjugated with glucuronic acid (t½ for linalool=23 min.). Linalool undergoes rapid oxidation (t½ for linalool=ll min.) in activated CYP-450 rat liver homogenate [Buck and Renwick,

1998]. Linalool, labeled with ^{14}C in positions 1 and 2, was orally administered to rats at a single dose of 500 mg/kg bw. The majority (55%) of the radioactivity was excreted in the urine as the glucuronic acid conjugate of linalool, while 23% was excreted as CO_2 in expired air, and 15% was excreted in the feces within 72 hours of dose administration. Only 3% of the radioactivity was detected in tissues after 72 hours, with 0.5% in the liver, 0.6% in the gut, 0.8% in the skin and 1.2% in the skeletal muscle [Parke et ***al.,*** 1974a] (see Figure 1). Reduction metabolites such as dihydro- and tetrahydrolinalool have been identified in the urine after administration of a single dose of linalool to rats [Rahman, 1974].

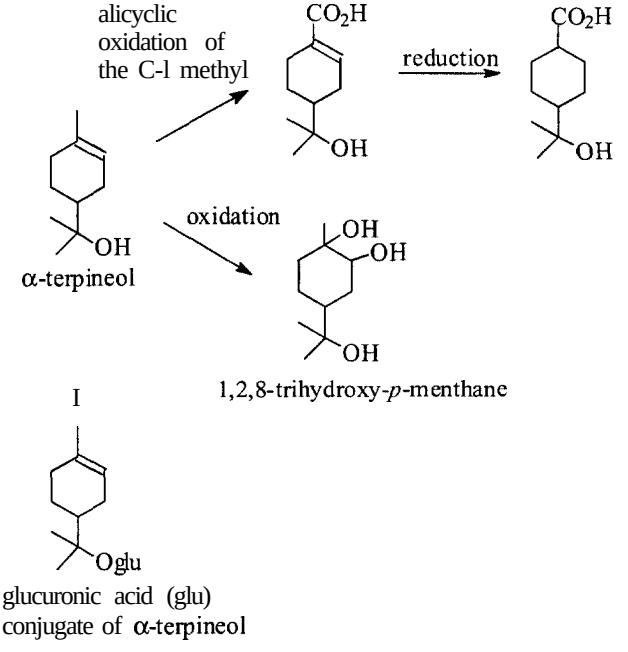
In a separate study, male rats (IISc strain) were given a daily 800 mg/kg bw oral dose of linalool for 20 days. Urinary metabolites formed by cytochrome P-450 (CYP450)-induced allylic oxidation of linalool included 8-hydroxylinalool and 8-carboxylinalool. CYP450 activity in the liver microsomes was increased approximately 50% after three days, but the activity decreased to control values after six days [Chadha and Madyastha, 1984]. Linalool administered daily to 4-week-old male Wistar rats by gavage at a dose of 500 mg/kg bw for 64 days did not induce CYP450 until the 30^{th} day of treatment [Parke et ***al.,*** 1974b].

The data suggest that glucuronic acid conjugation and excretion is the primary route of metabolism of linalool. Allylic oxidation becomes an important pathway only after repeated dosing. It has been suggested that the biotransformation of the diol metabolite of linalool to the corresponding aldehyde via the action of the nicotineamide adenine dinucleotide (NAD+) dependent enzyme alcohol dehydrogenase (ADH) is inhibited due to the bulky nature of the neighboring alkyl substituents and the substrate specificity of the enzyme [Eder et ***al.,*** 1982].

In a repeated dose study, male albino rats (IISc strain) were orally administered the alicyclic tertiary alcohol alpha-terpineol at a daily dose of 600 mg/kg bw for 20 days. Oxidation of the allylic methyl group was observed to yield the corresponding carboxylic acid, which to a small extent, was reduced to yield the corresponding saturated carboxylic acid [Madyastha and Srivatsan, 1988] (see Figure 2). alpha-Terpineol orally administered to rats increased the liver

microsomal CYP-450 content and activity of NADPH-cytochrome c reductase [Madyastha and Srivatsan, 1988] suggesting that oxidation is mediated by CYP-450.

Figure 2. Metabolism of alpha-terpineol in rats



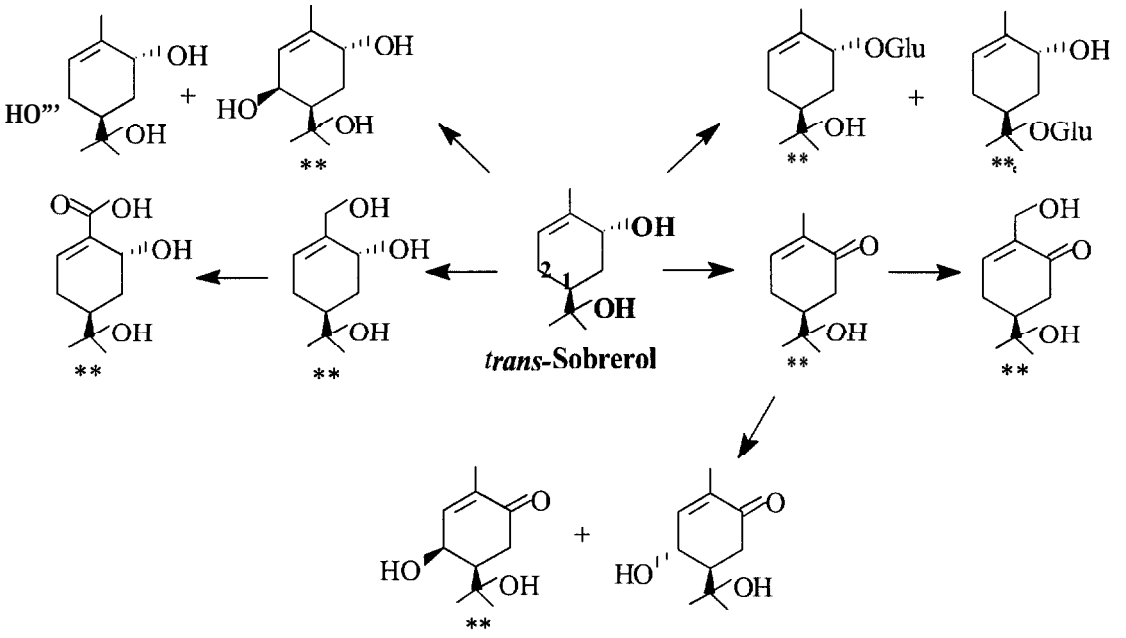
In a minor pathway, the endocyclic alkene of alpha-terpineol is epoxidized and then hydrolyzed to yield a trio1 metabolite 1,2,8-trihydroxy-*p*-menthane which also has been reported in humans following inadvertent oral ingestion of a pine oil disinfectant containing alpha-terpineol [Homing et ***al.,*** 1976]. It is expected that after single dose exposures, alpha-terpineol would undergo metabolism like linalool [Chadha and Madyastha, 1984], primarily glucuronic acid conjugation and excretion in the urine.

Bicyclic tertiary alcohols are conjugated with glucuronic acid and excreted primarily in the urine [Williams, 1959]. In rabbits the structurally related bicyclic tertiary alcohols thujyl alcohol (4-

methyl-1-(1-methylethyl)bicyclo[3.1.0]-hexan-3-ol) and beta-santenol (2,3,7-trimethylbicyclo[2.2.1]-heptan-2-ol) are conjugated with glucuronic acid [Williams, 1959].

In a metabolism study using the structurally related terpenoid tertiary alcohol *trans*-sobrerol (see Figure 3), in humans, dogs, and rats, ten metabolites were isolated in urine, eight of which were characterized in humans. Two principle modes of metabolism were observed, allylic oxidation of the ring positions and alkyl substituents, and conjugation of the tertiary alcohol functions with glucuronic acid. These metabolic patterns are common modes of converting tertiary [Ventura *et al.*, 1985] and secondary [Yamaguchi et *al.*, 1994] terpenoid alcohols to polar metabolites, which are easily excreted in the urine and feces (see Figure 3). Menthol forms similar oxidation and conjugation products in rats [Yamaguchi *et al.*, 1994].

Figure 3. Metabolism of *trans*-Sobrerol in Rats, Dogs, and Humans



** Isolated in human urine

In summary, esters of tertiary terpenoid alcohols are readily hydrolyzed in animals, including fish. Once hydrolyzed, the resulting alcohols undergo excretion unchanged or as the glucuronic acid conjugate. To a minor extent, CYP-450 mediated oxidation at the *omega* or *omega*- 1 position yields polar oxidized metabolites capable of excretion primarily in the urine.

2.8 Summary for Category Analysis

Based on the results of hydrolysis, the reactivity of linalool in aqueous media, and data on metabolism it is concluded that members of this chemical category exhibit similar chemical and biochemical fate. The esters are readily hydrolyzed to the corresponding alcohols, linalool and *alpha*-terpineol. Linalool is then partial converted to alpha-terpineol mainly under acidic conditions. Alicyclic and aliphatic tertiary alcohols are efficiently detoxicated by two principal pathways: conjugation primarily with glucuronic acid and excretion primarily in urine, and omega-oxidation to eventually yield diacids and their reduced or hydrated analogs. These polar metabolites will be efficiently excreted primarily in the urine either unchanged or as the glucuronic acid conjugates. The physiochemical and toxicological properties of these substances are consistent with their known reactivity and common metabolic fate.

3 Test Plan

3.1 Chemical and Physical Properties

3.1 .1 Melting Point

All the substances in this chemical category are liquids at ambient temperature. Calculated melting points indicate that alcohols and esters in this category would solidify below 23 °C [SRC]. The melting point of linalool is reported to be less than 20 °C [Givaudan Roure, 1991a] and the solidification temperature of the *d* and *l* forms of alpha-terpineol are reported to be 3 1 and 34.4 °C, respectively [SRC; Merck Index, 1997].

3.1.2 Boiling Point

Members of this chemical category are branched-chain acyclic or alicyclic tertiary alcohols and related esters. The molecular weights of the alcohols are within a narrow range (154 to 158 daltons). Therefore, it is not unexpected that their experimentally determined boiling points are also within a narrow range (196-230 "C). The alicyclic alcohols (e.g., alpha-terpineol) show slightly higher boiling points than their acyclic counterparts (e.g., myrcenol and linalool).

While only one of the reported boiling points was obtained according to a recent ASTM guideline, the consistency of the values reported by the Fragrance Materials Association (FMA) and standard reference sources (Merck Index, 1997) confirms their reliability. Linalool and plinol acetate (the corresponding ester of the major component of pinanol thermal rearrangement products) show relatively low boiling points of 198 [FMA] and 196 °C [Lurnsden, 1998], respectively, while alpha-terpineol exhibits a boiling point of 217-2 18 °C [FMA, FOA]. *cis*- and *trans*-Pinanol exhibit nearly identical boiling points (205-206 °C at 1 atmosphere pressure) [FMA]. The boiling point of pine oil, which is a mixture of mainly tertiary terpenoid alcohols, is in the range of 200-220 °C [FMA]. No boiling point is reported for the site-restricted peroxide pinane hydroperoxide. As a chemical intermediate, this thermally unstable substance is maintained at temperatures below 35 "C.

3.1.3 Vapor Pressure

The reported vapor pressures for linalool (0.05 mm Hg, 20 °C [Vuilleumeir et al., 1995] and 0.16 mm Hg (22-25 °C [Misra *et al.,* 1995]), alpha-terpineol (0.04 mm Hg, 22-25 °C [Misra *et al.,* 1995], and plinol 0.02 mm Hg, 22-25 °C [Misra *et al.,* 1995]) correlate well with calculated vapor pressures [FMA; SRC]. The calculated vapor pressure for all alcohols and esters in this group at 20 °C are in the range from 0.02 to 0.12 mm Hg [FMA]. No vapor pressure is reported for the site-restricted peroxide pinane hydroperoxide that is maintained in sealed containers at temperatures below 35 °C.

3.1.4 Octanol/Water Partition Coefficients

Measured log Kow values are available for four substances in this chemical category. Three alcohols, linalool, alpha-terpineol, and plinol exhibit log Kow values of 2.9 [Givaudan Roure, 1991b], 2.98 [Misra *et al.,* 1995], and 2.87 [Misra *et al.,* 1995], respectively. Higher log Kow values of 4.3 [Givaudan Roure, 1996a] and 4.09 [Lumsden, 1998] were reported for the more non-polar acetate esters, alpha-terpineol acetate and the related plinyl acetate, respectively.

The calculated log Kow values as reported by Syracuse Research Corporation (SRC), for eight tertiary terpenoid alcohols in this category are in the range from 2.87 to 3.47, are consistent with experimentally measured values (2.9 and 2.98). Likewise, calculated Kow values of 3.46 and 4.34 for linalyl acetate and *alpha-terpineol* acetate, respectively, agree with measured data for linalyl acetate (log Kow 2.9 [Proctor & Gamble, 1996]), alpha-terpineol acetate (log Kow=4.3 [Givaudan Roure, 1996a]) and for the structurally related ester, plinyl acetate (log Kow=4.09 [Lumsden, 19981).

3.1.5 Water Solubility

While the reported water solubilities were not obtained according to OECD guidelines, the solubility values increase with increased temperature and follow the same trend as measured partition coefficients. The values reported for linalool and alpha-terpineol at 4-8 °C (560 and

341 mg/L, respectively) and at 22-25 °C (867 and 716 mg/L, respectively) [Misra et al., 1995] show an expected increase in solubility. The value for linalool at 22-25 °C is consistent with the value reported for linalool at 20 °C (1450 mg/L) [Givaudan Roure, 1991a] and tetrahydrolinalool (700 mg/L, temperature not specified) [BBA, 1990]. The decreased solubility expected for esters in this category is realized with a solubility of 60.5 mg/L for plinyl acetate obtained from a recent GLP study [Lumsden, 1998] and 140 mg/L at 20 °C for linalyl acetate [Givaudan Roure, 1991 c]. Calculated values (ESPKOW) for the alcohols and esters in this category are in the range from 160 to 863 mg/L and less than 10 mg/L, respectively. The solubility of *cis-*, *tram-*, and unspecified 2-pinanol should exhibit essentially identical water solubilities (see robust summary for *cis*-2-pinanol).

3.1.6 New testing required

None

3.2 Environmental Fate and Pathways

3.2.1 Photodegradation

The calculated photodegradation half-lives for the ternary terpenoid alcohols and esters in this chemical category, are in the range fi-om 1.07 to 9.08 hours [AOPWIN]. Half-lives for alcohols (i.e., linalool and myrcenol) that contain a more reactive allyl alcohol moiety group are shorter than their saturated counterparts *(i.e.,* tetrahydrolinalool and dimyrcenol). Generally, more stable ternary alcohols in this category have longer half-lives than those for more reactive primary terpenoid alcohols *(i.e.,* citronellol, geraniol and nerol half-lives, 19 minutes to 1.3 hours). Calculated half-lives for linalool and alpha-terpineol(l.07 and 1.24 hours, respectively) are in the same range as their corresponding esters (1.10 and 1.35 hours, respectively). The decreased chemical reactivity of these tertiary alcohols, as compared to that of primary terpenoid alcohols, supports slightly longer photodegradation half-lives as predicted by the model.

The photodegradation model was not applied to the two mixtures in this chemical category. These mixtures are composed of tertiary alcohols and esters in this chemical category. As such, the photodegradation of the mixture is represented by the calculated photodegradation rates of its component alcohols and esters. Likewise, *cis*-, *trans*-, and unspecified 2-pinanol should exhibit nearly identical rates of photodegradation (see photodegradation robust summary for *cis*-2-pinanol).

3.2.2 Stability in Water

Hydrolysis data is available for the two acetate esters in this chemical category. Although hydrolysis is not possible for the tertiary alcohols in this category, it is possible for linalool to undergo acid-catalyzed ring closure to yield mainly alpha-terpineol and minor amounts of the primary terpenoid alcohols, geraniol and nerol. alpha-Linalyl acetate is calculated to have a half-life for hydrolysis of 174 days at pH 8 and 4.7 years at pH 7. Terpineol acetate is calculated to have a half-life for hydrolysis of 2.1 years at pH 8 and 21 years at pH 7. Experimental data indicate a more rapid rate of hydrolysis to yield the component alcohol. The mean half-life for hydrolysis of linalyl acetate at pH 1.2 was less than 2.5 minutes in aqueous artificial gastric juice in the absence or presence of hydrolytic enzymes. When the gastric juice was neutralized to pH 7.0 the half-life increased to 112 minutes. The hydrolysis half-life in aqueous artificial pancreatic fluid in the absence or presence of pancreatin enzyme at pH 7.5 was 97 and 83 minutes, respectively. Ester hydrolysis of linalyl acetate yields linalool and acetic acid. Linalool then partially rearranges to *alpha*-terpineol in both neutral and acidic media. The product ratio of linalool/*alpha*-terpineol was approximately 2: 1 at pH 1.2 or pH 7.5 [Buck and Renwick, 1999]. Mean half-life for alpha-terpineol acetate at pH 1.2 in the absence or presence of hydrolytic enzymes was 18.3 and 16.4 minutes, respectively. When pH was increased to 7.5, the half-life increased to 860 minutes. The hydrolysis half-life of *alpha*-terpineol acetate in aqueous artificial pancreatic fluid in the absence or presence of pancreatin enzyme at pH 7.5 was 358 and 371 minutes, respectively. Based on these data, either ester is anticipated to be completely hydrolyzed to the component acid and alcohol in aqueous media within one day.

3.2.3 Biodegradation

Two separate studies on linalool show this substance is readily biodegradable (*i.e.*, 97.1% biodegradation by OECD 301B and greater than 80% by modified MITI test at 28 days) [Quest, 1994d; Rudio, 1991]. The biodegradabilities of tetrahydrolinalool, myrcenol and dihydromyrcenol have also been measured. Tetrahydrolinalool and dihydromyrcenol were 103% and 72%, degraded, respectively, within 28 days in an OECD 301B assay [Quest, 1994b; Quest 1994c]. Myrcenol was 66% degraded in a 20-day closed bottle assay [UCC, 1986].

Two tertiary terpenoid esters have also been shown to be readily biodegradable. Linalyl acetate underwent 75% biodegradation after 28 days in an OECD modified MITI test [Quest, 1991] and alpha-terpineol acetate exhibited 87.3% and 63% biodegradation in an OECD 301B test [Quest, 1994a] and an OECD 301F test [Givaudan Roure, 1996b], respectively. While no studies are available on *cis*- or *trans*-2-pinanol, there is no reason to believe that these materials will not also be readily biodegradable. The site-restricted chemical intermediate 2-pinanol hydroperoxide will decompose to 2-pinanol [Schmidt and Fisher, 1959] in the environment and thus should be considered to be readily biodegradable. Pliny1 acetate was only 6% degraded in a 28-day OECD 301F test [Mead, 1997b]. This result is inconsistent with the biodegradability results for linalyl acetate and alpha-terpineol acetate in which ultimate biodegradability was observed. In an activated sludge respiration inhibition test the no effect concentration (NOEC) for plinyl acetate was determined to be 100 mg/L [Mead, 1997c]. In an older biodegradability study reporting limited data [Ryckman, 1966], pine oil underwent appreciably biodegradation as measured by a 5-day BOD (0.8 mg/ml) 50% of the measured COD (1.6 mg/ml).

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Mackay, 1991]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log Kow. Where measured values were available, these were used, but where

they were not, calculated data from the EPIWIN series of programs were used. Based on the hydrolysis of the esters to yield component tertiary alcohols and comparable physiochemical properties of the tertiary alcohols, it is not unexpected that the alcohols in this category would exhibit similar distribution in the environment. The distribution of 2-pinanol in the environment is represented by the fugacity calculation for its *cis*- and *trans*-isomers.

The significance of these calculations must be evaluated in the context that the substances in this chemical category are products of plant biosynthesis and are, therefore, ubiquitous in the environment. Most have been shown to be readily and/or ultimately biodegradable, and the remainder would be expected to behave similarly in the environment. The model does not account for the influence of biogenic production on partitioning in the environment nor does it take into account biodegradation. Therefore, the relevance of fugacity calculations for these substances is highly questionable.

3.2.5 New testing required

None

3.3 Ecotoxicity

3.3.1 Acute Toxicity to Fish

Calculated ECOSAR values and nine studies of acute fish toxicity are available for this chemical category. Calculated 96 hr ECOSAR values are in the range from 0.60 mg/L for linalool (calculated as a vinyl alcohol) to 18.2 mg/L for *cis* or *trans*-2-pinanol. With few exceptions, the calculated LC50 values were significantly less than actual experimental values. The 96 hr LC50 values for pine oil in bluegill and rainbow trout in flow-through systems were reported to be 53 and 18 mg/L, respectively [Graves *et al.*, 1994a, 1994b]. In other acute toxicity experiments with pine oil, the 48 hr LC50 value was determined to be 58-59 mg/L for bluegill [Ryckman, 1965] and the 96 hr LC50 value was 71 mg/L in juvenile rainbow trout. The later result was obtained in a range finding study that was used to determine nominal concentrations for a

subsequent 96 hr LC50 flow-through test in the same species [Graves et ***al.,*** 1994b]. Calculated 96 hr LC50 values for 2-pinanol and ***alpha-terpineol,*** components of pine oil, are in the same range, 18.2 mg/L and 13.7 mg/L, respectively. An isomer of linalool, isolinalool, exhibited a 96 hr LC50 of 33 mg/L in juvenile rainbow trout [LeLievre, 1990a].

Good correlation exists between the experimental and calculated 96 hr LC50 values for plinyl acetate and myrcenol. The 96 hr LC50 value for rainbow trout exposed to plinyl acetate is reported to be 11.0 mg/L [Wetton, 1997a] while the calculated ECOSAR value is 17.4 mg/L. The measured 96 hr LC50 value for myrcenol in fathead minnows was reported to be 3.7 mg/L [UCC, 1986], which is closely correlated with the calculated 96 hr LC50 of 4.85 mg/L [ECOSAR]. This latter study may be considered of limited value for assessing acute fish toxicity in that minimal aeration was supplied when the dissolved oxygen fell below 4 mg/L.

In a static system test ***in*** which ***alpha-terpineol*** was used in conjunction with an emulsifier (10%), the 96 hr LC50s for Coho salmon and rainbow trout were reported to be 6.8 and 6.7 mg/L, respectively [Stroh et ***al.,*** 1998]. The calculated 96 ***hr*** LC50 value for *alpha*-terpineol is 13.7 mg/L.

Although there are sufficient data on this chemical category, conducting an assay with linalool following OECD Guideline 203 would provide key data in order to validate the QSAR algorithm and to help determine whether this material should be considered as a vinyl alcohol or as a neutral organic.

3.3.2 Acute Toxicity to Aquatic Invertebrates

In addition to ECOSAR calculated 48 hr LC50 values, experimental data from 4 studies are available for members of this chemical category. Similar to fish acute toxicity data, 48 hr LC50 values are typically lower by an order of magnitude than experimental values. Calculated values are in the range from 4.4 to 20.6 mg/L for tertiary terpenoid alcohols and 1.04 to 1.16 for tertiary terpenoid esters [ECOSAR]. The 48 hr LC50 for myrcenol with *D. **magna*** was 36 mg/L [UCC, 1986] while the calculated value was 5.75 mg/L. The 48 hr EC50 values with *D.*

magna were reported to be 7.0 mg/L for plinyl acetate [Wetton, 1997b] and 24 mg/L for pine oil [Graves ***et al.,*** 1994c]. Both studies were performed using OECD 202 guidelines. The value of 24 mg/L for pine oil is in good agreement with the ECOSAR calculated value of 15.7 mg/L for alpha-terpineol, a principal component of pine oil. The reported 48 hr EC50 level for isohnalool ***with Daphnia*** *pulex* was reported to be 47 mg/L [LeLievre, 1990b]. Given the available data for members of this chemical category, the approximate range of acute toxicity to aquatic invertebrates, particularly ***D. magna,*** is expected to be in the range of 10-50 mg/L. Given the low calculated LC50 values for tertiary terpenoid esters, it is recommended that an additional LC50 assay be performed for ***D. magna*** with linalyl acetate using an OECD 202 Guideline.

3.3.3 Acute Toxicity to Aquatic Plants

In a manner similar to fish and invertebrate toxicity, calculated 96 hr EC50 values for plant ***(i.e.,*** green algae) are lower than experimental values. Calculated values are in the range from 0.14 to 13.6 mg/L with the majority of values falling in the 3-5 mg/L range for the tertiary alcohols and less than 1 mg/L for tertiary terpenoid esters. Experimental data from 4 studies are available for members of this chemical category and EC50 values are consistently higher than the calculated values. Both linalool and ***alpha-terpineol*** were subjected to a plate inhibition assay with Chlorella pyrenoidosa using test concentrations of 100, 1000 and 10,000 mg/L [Ikawa ***et al.,*** 1992]. ***alpha-Terpineol*** showed no inhibition at any concentration, while linalool showed only mild reduction in colony density as reflected in lawn color at the highest concentration. In a test following OECD 201 Guideline, the reported algal 72 hr EC50 value was greater than 15 mg/L for plinyl acetate in Scenedesmus subspicatus [Mead, 1997a]. A 96 hr NOEC of 3.3 mg/L was reported for isolinalool in Selenastrum capricomutum [Giddings, 1990]. The three measured EC50 values for linalool, ***alpha-terpineol,*** and plinyl acetate and the single NOEC concentration for isolinalool are all greater than their respective calculated EC50 values. The current database indicates that the EC50 values for members of this chemical category would be in the range of greater than 10-20 mg/L. Given the limited database of information for esters and pinanol

derivatives in this chemical category, it is recommended that acute EC50 assays be performed for linalyl acetate and 2-pinanol using OECD Guideline 20 1.

3.3.4 New Testing Required

Acute toxicity to fish by OECD guideline 203 for linalool;

Acute toxicity to aquatic invertebrates by OECD guideline 202 for linalyl acetate;

Acute toxicity to aquatic plants by OECD Guideline 201 for linalyl acetate and 2-pinanol.

3.4 Human Health Data

3.4.1 Acute Toxicity

Oral and dermal LD50 values for members of this chemical category indicate a low order of both oral and dermal toxicity. All rabbit dermal, and mouse and rat oral LD50 values exceed 2000 mg/kg with the majority of values greater than 5000 mg/kg [Baker et al., 1976; Cerven, 1985a, 1985b; Deichmann, 1961; Jenner, 1964; Johnson, 1976; Moreno, 1972a, 1972b, 1973, 1976; Palanker, 1979; Robbins, 1994; Sanders, 1997, Fogelman, 1970].

3.4.2 *In* vitro Genotoxicity

Mutagenicity/genotoxicity testing has been performed on six members of this chemical category, including a complete battery of *in vitro* genotoxicity tests using linalool. Linalool [Heck *et al.,* 1989, Ishidate *et al.,* 1984; Florin *et al.,* 1980; Rockwell and Raw, 1979] and tetrahydrolinalool [Wild *et al.,* 1983] were inactive *in Salmonella typhimurium (SAL) strains* TA92, TA94, TA98, TA100, TA1535, TA1537, and TA1538 with and without S-9 metabolic activation. Linalool did not induce chromosomal aberrations when incubated with Chinese hamster fibroblast cells at a maximum concentration of 0.25 mg/ml [Ishidate *et al.,* 1984], nor did it induce unscheduled DNA synthesis (UDS) in rat hepatocytes at concentrations up to 50 nl/ml [Heck *et al.*, 1989].

When incubated with *E. coli WP2 uvrA* at concentrations of 125- 1000 mg/plate, linalool was negative for mutation induction [Yoo, 1986]. When incubated with *Bacillus subtilis* H 17 (rec+) and M45 (rec-), linalool was negative at 17 µg [Oda *et al.,* 1978] but positive at 10 µl/disk

[Yoo, 1986]. The latter result occurred in a study in which the majority of alcohols and aldehydes were positive while non-oxidizable substrates (esters, ketones, lactone, and acid) were negative. The positive responses in that study [Yoo, 1986] are clearly contradicted by the results of the same assay by other researchers [Oda *et al.,* 1978]. In a mouse lymphoma assay, linalool was negative in L5 178Y TK+/- cells with S-9 metabolic activation at 200 nl/ml but weakly positive without S-9 activation at 150 nl/ml [Heck *et al.,* 1989].

Linalyl acetate and *alpha-terpineol were inactive in Salmonella typhimurium (SAL) strains* TA98, TAlOO, TA1535, TA1537, and TA1538 with and without S-9 metabolic activation [Heck et *al.,* 1989; Ishidate *et al.,* 1984; Florin *et al.,* 1980]. *p*-Menth-8-en-1-ol (*alpha*-terpineol) was inactive in SAL strains TA98 and TAlOO with S-9 metabolic activation [Rockwell and Raw, 1979]. When incubated with *Bacillus subtilis* H17 (rec+) and M45 (rec-), hnalyl acetate and terpinyl acetate were negative at 18 µg and 19 µg, respectively [Oda *et al.,* 1978].

In an *in vivo-in vitro* study designed to investigate the mutagenicity of the metabolites of linalool and alpha-terpineol, Sprague-Dawley rats were administered a single dose of 0.5 ml (452 mg) of linalool or alpha-terpineol by gavage and the urine was collected for 24-hours. The urine (500 ul) was hydrolyzed *with beta-glucuronidase.* Hydrolyzed and unhydrolyzed urine samples, ether extracts of the urine, and aqueous fractions of the urine-ether extracts were then separately incubated with SAL strains TA98 and TAlOO without S9 activation. Linalool, *alpha*-terpineol, and none of the urinary solutions isolated from the urine of rats given 452 mg doses of either linalool or *alpha-terpineol* showed any evidence of mutagenicity in either TA98 or TAlOO without metabolic activation [Rockwell and Raw, 1979].

In nineteen separate *in vitro* tests on the mutagenicity and genotoxicity of terpenoid tertiary alcohols and related esters, all but two were negative. One of the positive results for linalool was observed in a rec assay using differences in growth rates *in two strains* of *Bacillus subtilis* as a measure of DNA changes [Yoo, 1986]. In contrast, no evidence of mutagenicity was observed in the same test at a higher concentrations [Oda *et al.,* 1978] nor was DNA damage observed

in a rat hepatocyte UDS assay [Heck et *al.,* 1989]. The authors of the mouse lymphoma assay [Heck et *al.,* 1989], which gave a weak positive result for linalool, emphasized that positive results in this assay are commonly observed for polar substances in the absence of S-9 and may be associated with changes in physiologic culture conditions (pH and osmolality).

3.4.3 *In* vivo Genotoxicity

In a standardized mouse micronucleus assay, male and female CD- 1 mice were given single intraperitoneal injections of 116, 578, or 1155 mg/kg pine oil and bone marrow samples were retrieved at 24, 48, and 72 hours. There was no evidence of an increased incidence of micronucleated polychromatic erythrocytes at any dose level tested [Putman, 1987].

Based on a weight of evidence evaluation of the available in *vitro* and *in vivo* mutagenicity and genotoxicity assays on terpenoid tertiary alcohols and related esters, this group of flavoring substances would not be expected to exhibit a low genotoxic potential *in vivo.*

3.4.4 Repeat Dose Toxicity

3.4.4.1 Linalool

In a safety evaluation study, a 50/50 mixture of linalool and citronellol was fed to male and female rats (number and strain not specified) in the diet. The daily intake was calculated to be 50 mg/kg bw of each. Measurements of hematology, clinical chemistry, and urinalysis at weeks 6 and 12 showed no statistically significant differences between test and control groups. Histopathology revealed no dose-related lesions. A slight retardation of growth was observed in males only, but was concluded by the authors to be biologically insignificant [Food and Drug Research Lab., 1958].

3.4.4.2 Linalyl esters

For 12 weeks, a mixture of linalyl acetate, linalyl isobutyrate and geranyl acetate was added to the diet of male and female rats (strain not specified) at levels calculated to result in average daily intakes of 24, 27, or 48 mg/kg bw, respectively. Measurements of hematology, clinical

chemistry, and urinalysis at weeks 6 and 12 showed normal values. Histopathology revealed no dose-related lesions. A slight retardation of growth was observed in females only, but was concluded by the authors to be biologically insignificant [Trubeck Laboratories, 195 8].

Four groups of 10 male and 10 female Osborne-Mendel rats were fed the structurally related ester of linalool, linalyl isobutyrate, at dietary concentrations of 0, 1000, 2500, or 10,000 ppm for 18 weeks. These levels were calculated [FDA, 1993] to provide estimated daily intakes of 0, 50, 125, or 500 mg/kg bw, respectively. Body weight, hematology, and gross pathology were performed on all test animals, and microscopic examination was performed on the high dose group only. There were no differences between treated and control animals in the parameters evaluated [Hagan et al., 1967].

In a co-carcinogenicity study, 3 mg of linalyl acetate was applied either alone or in conjunction with the carcinogen benzo-[a]-pyrene to the skin of female ICR/Swiss Ha mice (20/group) 3 times per week for 67 weeks. Mice showing tumors for 30 days or more, greater than 1 mm in size, were counted. There was no increase in the incidence of tumors in mice treated with linalyl acetate itself or with benzo-[a]-pyrene [Van Duuren *et al.,* 1971].

3.4.4.3 alpha-Terpineol acetate

Ten (10) male and 10 female weanling Osborne-Mendel rats were fed terpinyl acetate in the diet for 20 weeks at concentrations of 0, 1000, 2500 or 10,000 ppm [Hagan *et al.,* 1967]. These dietary levels were calculated [FDA, 1993] to result in daily intakes of 0, 50, 125 and 500 mgikg bw, respectively. All animals were examined for growth, hematology, and macroscopic changes in the tissues. Microscopic examination was performed on 6-8 male and female animals in the high dose and control groups. No statistically significant adverse effects were reported [Hagan *et al.,* 1967].

3.4.4.4 2,6,6-Trimethylbicyclo[3.1.1]heptan-2-ol thermal rearrangement products (structurally related substance - plinyl acetate)

Following a range- finding, repeat-dose toxicity study [Thomas, 1997], groups of male and female Sprague-Dawley rats were administered 0, 15, 150,0r 1000 mg/kg/d of plinyl acetate by gavage daily for 28 days. Based on the results of daily observation for clinical signs, weekly measurement of body weight and food consumption, hematologkal examinations, blood chemical determinations, measurement of organ weights and gross and histopathological examination, it was concluded that 15 mg/kg/d was a NOAEL for plinyl acetate in male and female rats. At the 150 mg/kg/d level, a statistically significant increase in liver and kidney weights was accompanied by histopathological changes including accumulation of globular eosinophilic material in male kidney, and centrilobular hepatocyte enlargement in male rats [Thomas, 1997].

3.4.5 Reproductive Toxicity

Four groups of 10 virgin Crl CD rats were administered 0, 250, 500, or 1000 mg/kg bw of an essential oil (coriander oil) known to contain 73% linalool by mass. The test material was given by gavage once daily, 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days. Maternal indices monitored included twice daily observation, measurement of body weights, food consumption, duration of gestation, and fertility parameters (mating and fertility index, gestation index, number of offspring per litter). Offspring indices included daily observation, clinical signs, examination for gross external malformations, and measurement of body weight. Maternal effects reported included increased body weight and increased food consumption at 250 mg/kg/d, a non-statistically significant decrease in body weight and food consumption and decreased gestation index and decreased length of gestation at 500 mg/kg/d, and a statistically significant decrease in body weight and food consumption, statistically significant decrease in gestation index, length of gestation, and litter size at 1000 mg/kg/d. The only effect on pups was a decrease in viability of pups at the highest dose level. The authors concluded that there were no effects observed in the dams at the low dose of 250 mg/kg bw/d or in the offspring at the

250 and 500 mg/kg bw/d levels. The authors concluded that the maternal NOAEL was 250 mg/kg/d and the developmental NOAEL was 500 mg/kg/d [Vollmuth *et al.,* 1995].

Four groups of 10 virgin Crl CD rats were administered 0, 375, 750, or 1500 mg/kg bw of an essential oil (cardamom oil) known to contain greater than 65 % tertiary terpenoid alcohols with 5 1% alpha-terpineol acetate by mass. The test material was given by gavage once daily, 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days. Maternal indices monitored included twice-daily observation, measurement of body weights, food consumption, duration of gestation, and fertility parameters (mating and fertility index, gestation index, number of offspring per litter). Offspring indices included daily observation, clinical signs, examination for gross external malformations, and measurement of body weight. Maternal observations included a non-statistically significant decrease in body weight gain and food consumption at 375 mg/kg/d. Mortality, clinical signs, a statistically significant decrease in body weight gain and food consumption, and gross lesions at necropsy were seen at 750 and 1500 mg/kg/d. The only effects on pups were a reduced body weight gain in pups at 750 and 1500 mg/kg/d and increased mortality at 1500 mg/kg/d. The authors concluded that there were no significant adverse effects in the dams or offspring at the 375 mg/kg/d dose. A maternal NOEL was reported to be less than 375 mg/kg/d based on reduced body weight gain and food consumption at 375 mg/kg/d and a developmental NOAEL was reported to be 375 mg/kg/d [Vollmuth *et al.*, 1995].

3.4.6 Developmental/Teratogenicity Toxicity

In addition to the two developmental/reproduction screening studies above, a range-finding study and follow-up teratology study was performed with pine oil. Pregnant Crl:CD(SD) BR rats were given 0, 50, 100, 500, 750,or 1000 mg/kg/d by gavage in corn oil on days 6 to 20 of gestation. Laparotomies were performed, corpora lutea were counted, and the uterus of each rat was removed, weighed and then examined for number, placement and viability of implantations. Live fetuses were weighed, sexed and gross external alternations were identified.

Soft tissue and skeletal evaluations were not performed in the pilot study. There were no deaths or abortions during the course of this study. Necropsy revealed no gross lesions. Maternal effects included local alopecia, decreased body weight gain and food consumption for the 3 highest dose levels. At 750 and 1000 mg/kg, average gravid uterine weight was reduced. In fetuses, decreased body weight was observed at dose levels of 100 mg/kg and above, and at dose levels of 500 and above there was a slight increase in average number of resorptions/litter [Dearlove, 1987].

In the follow-up teratology study, pregnant Crl:CD(SD) BR rats were given 0, 50, 600, or 1200 mg/kg/d by gavage in corn oil on days 6 to 20 of gestation. Rats were examined for pregnancy, number and placement of implantations, early and late resorptions, live and dead fetuses, and number of corpora lutea. Fetuses were subsequently weighed and evaluated for both soft and hard tissue abnormalities. At 600 and 1200 mg/kg/d, decreased food consumption, body weight gains, excess salivation, alopecia, ungroomed coat, ataxia, decreased motor activity, impaired righting reflex and urine stained abdominal fur were observed Six of the 25 rats in 1200 mg/kg dose group died and necropsies revealed that adrenal weights were significantly increased in these rats. At 1200 mg/kg/d, fetuses exhibited increased incidences of delayed ossification, delayed brain development, decreased weights, increased embryo -fetal mortality, and sunken eye bulge with associated soft and hard tissue findings, a dose that also resulted in maternal death and a low incidence of embryo-fetal death (resorption). The maternal and developmental NOEL for pine oil was greater than 50 mg/kg/d but less than 600 mg/kg/d [Parent, 1988].

3.4.7 New Testing Required

None

3.5 Test Plan Table

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 78-70-6 Linalool (3,7-dimethyl-1,6-octadien-3-ol)	A	A, Calc	A, Calc	A, Calc	A, Calc
CAS No. 78-69-3 Tetrahydrolinalool (3,7-Dimethyl-3-Octanol)	Calc	A	Calc	Calc	A, Calc
CAS No. 543-39-5 Myrcenol (2-methyl-6-methylene-7-octen-2-ol)	Calc	A	Calc	Calc	Calc
CAS No. 18497-58-8 Dihydromyrcenol (3,7-dimethyl-7-octen-2-01)	Calc	A, Calc	Calc	Calc	Calc
CAS No. 115-95-7 Linalyl acetate (3,7-dimethyl-1,6-octadien-3-yl acetate	Calc	A, Calc	Calc	A, Calc	A, Calc
CAS No.98-55-5 alpha-Terpineol (p-menth-1-en-8-	Calc	A	A, Calc	A, Calc	A, Calc
CAS No. 80-26-2 alpha-Terpinyl acetate (p-menth-1-en-8-yl acetate)	Calc	A	Calc	A, Calc	Calc
CAS No. 125252-49-5 2,6,6-Trimethylbicyclo[3.1.1]heptan-2-01 thermal rearrangement products	NA	A	A	A, Calc	A, Calc
CAS No. 4948-28-l *cis*-2-Pinanol	NA	A, Calc	Calc	Calc	Calc
CAS No 473-54-l Pinanol	Calc	A	Calc	Calc	R
CAS No. 4948-29-2 trans-2-Pinanol	NA	A, Calc	Calc	Calc	R
CAS No. 28324-52-9 2-Pinanol hydroperoxide	NA	NA	NA	Calc	Calc
CAS No. 8002-09-3 Pine oil	NA	A	Calc	Calc	A

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
CAS No. 78-70-6 Linalool (3,7-dimethyl-1,6-octadien-3-ol)	Calc	A	A	Calc
CAS No. 78-69-3 Tetrahydrolinalool (3,7-Dimethyl-3-Octanol)	Calc	NA	A	Calc
CAS No. 543-39-5 Myrcenol (2-methyl-6-methylene-7-octen-2-ol)	Calc	NA	R	Calc
CAS No. 18497-58-8 Dihydromyrcenol (3,7-dimethyl-7-octen-2-ol)	Calc	NA	A	Calc
CAS No. 115-95-7 Linalyl acetate (3,7-dimethyl-1,6-octadien-3-yl acetate	Calc	A	A	Calc
CAS No.98-55-5 alpha-Terpineol (p-menth-l-en-%01)	Calc	NA	R	Calc
CAS No. 80-26-2 alpha-Terpinyl acetate (p-menth-1-en-8-yl acetate)	Calc	A	A	Calc
CAS No. 125252-49-5 2,6,6-Trimethylbicyclo[3.1.1]heptan-2-01 thermal rearrangement products	R	NA	A	Calc
CAS No. 4948-28-l cis-2-Pinanol	Calc	NA	R	Calc
CAS No 473-54-l Pinanol	R	NA	R	R
CAS No. 4948-29-2 *trans*-2-Pinanol	R	NA	R	Calc
CAS No. 28324-52-9 2-Pinanol hydroperoxide	Calc	NA	R	Calc
CAS No. 8002-09-3 Pine oil	R	NA	A	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 78-70-6 Linalool (3,7-dimethyl-1,6-octadien-3-ol)	R,Calc, T	R	A. Calc
CAS No. 78-69-3 Tetrahydrolinalool (3,7-Dimethyl-3-Octanol)	R, Calc	R, Calc	R, Calc
CAS No. 543-39-5 Myrcenol (2-methyl-6-methylene-7-octen-2-ol)	A, Calc	A, Calc	R, Calc
CAS No. 18497-58-8 Dihydromyrcenol (3,7-dimethyl-7-octen-2-ol)	R, Calc	R, Calc	R, Calc
CAS No. 115-95-7 Linalyl acetate (3,7-dimethyl-1,6-octadien-3-yl acetate	R, Calc	T, Calc	T, Calc
CAS No.98-55-5 alpha-Terpineol (p-menth-1-en-8-01)	A, Calc	R, Calc	A, Calc
CAS No. 80-26-2 alpha-Terpinyl acetate (p-menth-1-en-8-yl acetate)	R, Calc	R, Calc	R, Calc
CAS No. 125252-49-5 2,6,6-Trimethylbicyclo[3.1.1]heptan-2-01 thermal rearrangement products	A, Calc	A	A
CAS No. 4948-28-l cis-2-Pinanol	R, Calc	R, Calc	R, Calc
CAS No 473-54-l 2-Pinanol	R	R	T
CAS No. 4948-29-2 *trans*-2-Pinanol	R, Calc	R	R
CAS No. 28324-52-9 2-Pinanol hydroperoxide	R, Calc	R, Calc	R
CAS No. 8002-09-3 Pine oil	A, Calc	A	R

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
CAS No. 78-70-6 Linalool (3,7-dimethyl-1,6-octadien-3-ol)	**A**	A	**R**	**A**	**A**	**R**
CAS No. 78-69-3 Tetrahydrolinalool (3,7-Dimethyl-3-Octanol)	**A**	**A**	**R**	R	**R**	**R**
CAS No. 543-39-S Myrcenol (2-methyl-6-methylene-7-octen-2-ol)	**A**	**R**	R	R	R	**R**
CAS No. 18497-58-8 Dihydromyrcenol (3,7-dimethyl-7-octen-2-ol)	**A**	**R**	**R**	**R**	**R**	**R**
CAS No. 115-95-7 Linalyl acetate (3,7-dimethyl-1,6-octadien-3-yl acetate	**A**	**A**	**R**	**A**	**R**	**R**
CAS No.98-55-5 alpha-Terpineol (p-menth-1-en-S-01)	**A**	**A**	**R**	**R**	**R**	**R**
CAS No. 80-26-2 *alpha*-Terpinyl acetate (p-menth-1-en-8-yl acetate)	**A**	**A**	R	**A**	**A**	**R**
CAS No. 125252-49-s 2,6,6-Trimethylbicyclo[3.1.1]heptan-2-ol thermal rearrangement products	**A**	**A**	**R**	**A**	**R**	**R**
CAS No. 4948-28-l *cis*-2-Pinanol	**A**	**R**	**R**	**R**	**R**	R
CAS No 473-54-l Pinanol	**R**	R	R	R	R	**R**
CAS No. 4948-29-2 *trans*-2-Pinanol	**R**	**R**	**R**	**R**	**R**	**R**
CAS No. 28324-52-9 2-Pinanol hydroperoxide	**R**	**R**	**R**	**R**	**R**	**R**
CAS No. 8002-09-3 Pine oil	**A**	**A**	**A**	R	R	**A**

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
T	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
0	Other

4 References for Test Plan and Robust Summaries

Abas R. and Hayton W. L. (1997) A physiologically based pharmacokinetic and pharmacodynamic model for paraoxon in rainbow trout. *Toxicol. Appl. Pharmacol.,* 145, 192-201.

Baker R. G., Mastri, C. W., Kinoshita, F. K and Keplinger, M. L. (1976) Report to Hercules Inc. on acute toxicity studies with Hercules 70L pine oil disinfectant by Industrial Bio-Test Laboratories, Inc. Project No. 76-05.

Barron M. G., Charron K. A., Stott W. T., Duvall S. E. (1999) Tissue carboxylesterase activity of rainbow trout. *Environmental Toxicology and Chemistry,* 18, 2506-251 1.

Blagden S. M. (1997). Plinyl Acetate: Acute Inhalation Toxicity (Nose Only) Study in the Rat. Safepharm Laboratories Ltd. SPL Project No. 1044/017.

Blumenthal J. H. (1966) International Flavors & Fragrances Inc., US 3413364 (1966). *Chem. Abstr.* **70**, 47639a.

Boone J. S. and Chambers, J. E. (1996) Time course of inhibition of cholinesterase and aliesterase activities and nonprotein sulfhydryl levels following exposure to organophosphorous insecticides in mosquito fish. (Gambusia affinis). *Fundam Appl Toxicol. 29, 203-207.*

Brose T., Pritzkow, W. and Thomas, G (1992) Studies on the oxidation of *cis*- and *trans*-Pinane with molecular oxygen. *J. Prakt.* Chem. 334, 403409.

Buck N. R. and Renwick, A. G. (1998) Report on the behaviour of linalool in artificial gastric and pancreatic juices. Unpublished report to FEMA.

Buck N. R. and Renwick, A. G. (1999) Report on the behaviour of linalool in artificial gastric and pancreatic juices. Unpublished report to FEMA.

Bush Boake Allen (BBA) (1990). Unpublished report to RIFM.

Canova L. A. (1977) SCM Corp. US 4018842 (1976) *Chem Abstr. 87,* 23560t.

Cerven B., Moreno, 0. M. and Althenbach, E. (1985a) Single dose oral toxicity in rats (20% Pine Oil Disinfectant. Report to Hercules Inc. by M. B. Research Laboratories. Project No. MB 85-7546 A.

Cerven B., Moreno, 0. M. and Althenbach, E. (1985b) Acute dermal toxicity in rabbits (20% Pine Oil Disinfectant. Report to Hercules Inc. by M. B. Research Laboratories. Project No. MB 85-7546 B.

Chadha A. and Madyastha K.M. (1984) Metabolism of geraniol and linalool in the rat and effects on liver and lung microsomal enzymes. *Xenobiofica 14, 365374.*

Chemical Sources Manufacturers' Association (CSMA) (2000). Private Communication to FFHPVC. Unpublished report.

Curren R. D. (1987) Unscheduled DNA Synthesis in Rat Primary Hepatocytes with a Condirmatory Assay. Microbiological Associate, Inc. Report to Chemical Specialties Manufacturers Association. Lab. Study No. T5366.380016.

Davis K. (2000) Constituents for two lots of pine oil blends CSMA 1687 and 021494. Private communication to FFHPVC. Unpublished report.

Dearlove G. E. (1987) Dosage-Range Developmental Toxicity Study of Pine Oil Blend 1687 Administered Orally Via Gavage to Crl:CD(SD)BR Presumed Pregnant Rats (Pilot Study). Appendix IV to Parent, R.A. (1988). Teratogenicity Study in Albino Rats with Pine Oil Blend. A Report Submitted by Pine Oil Joint Venture to Chemical Specialties Manufacturers Association.

Deichmann W. B. (1961) Report on the determination of the approximate lethal oral dose in the rat of compounds submitted by the Hercules Powder Co.

Diliberto J. J., Usha, G. and Birnbaum, L. S. (1988) Disposition of citral in male Fischer rats. Drug *Metabolism and Disposition* 16, 721-727.

Eder E., Henschler D., Neudecker T. (1982) Mutagenic properties of allylic and α,β-unsaturated compounds: Considerations of alkylating mechanisms. *Xenobiotica* ***12, 831-848.***

Food and Agricultural Organization of the United States (FAO) (1997) Compendium of food additive specifications addendum 5.

Flavor and Extract Manufacturers' Association (FEMA) (1998) Report on the metabolism of linalyl acetate in rat tissue & intestinal homogenates. Unpublished report.

Florin I., Rutberg L., Curvall M. and Enzell CR. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology 15, 219-232.*

Fogelman R.W. (1970) Acute dermal toxicity study in rabbits. Unpublished report to RIFM.

Food and Drug Administration (FDA) (1993) Priority-based Assessment of Food Additives (PAFA) Database. Center for Food Safety and Applied Nutrition. p. 58.

Food and Drug Research Laboratories (1958) Toxicological Screening of Components of Food Flavors Class VI. Citronellol and Linalool.

Fragrance Materials Association (FMA) Reported values for boiling point.

Giddings J. (1990). 2,CDimethyl 2,7-octadien-4-ol - acute toxicity to freshwater green alga (Selenastrum capricornutum) under static conditions. Springborn Lab. Inc. Report Submitted to SCM Glidco Organics. SLI Report #390-10-3497.

Givaudan Roure (1991a) Determination of ready biodegradability of linalool. Unpublished report

Givaudan Roure (1991 b) Determination of the partition coefficient (octanol/water) of linalool. Unpublished report.

Givaudan Roure (1991c) Determination of the ready biodegradability of linalyl acetate synthetic, Unpublished report.

Givaudan Roure (1996a) Partition coefficient n-octanol/water of alpha-terpineol acetate. Unpublished report.

Givaudan Roure (1996b) Ready biodegradability of alpha-terpineol acetate. Unpublished report.

Gopalakrishnan M., and Narayan C. S. (1991) Supercritical carbon dioxide extraction of cardamom, *J. Agric. Food Chem. 39, 1976-1978.*

Goldblatt L. A. (1951) American Turpentines in The Essential Oils, Volume Six, Individual Essential Oils of the Plant Families. Publishers: Van Nostrand Reinhold Co., Litton Educational Publishing, Inc. New York, NY.

Gorman, C. R. (1976) Crude Pinanols (8N4) - Composition/identification. SCM Glidden-Durkee Project Report No. 840.

Graves W. C. and Swigert, J. P (1994a) Pine Oil: 96-hours flow-through acute toxicity test with bluegill (Lepomis macrochirus). Wildlife International Ltd Report to The CSMA Pine Oil Joint Venture. Lab Project ID/Study Number 274A-101.

Graves W. C. and Swigert, J. P (1994b). Pine Oil: 96-Hours Flow-through Acute Toxicity Test with the rainbow trout (Oncorhynchus mykiss). Wildlife International Ltd Report to The CSMA Pine Oil Joint Venture. Lab Project ID/Study Number 274A-102.

Graves W. C. and Swigert, J. P (1994c) Pine Oil: Forty-eight hours flow-through acute toxicity test with the cladoceran (Daphnia magna). Wildlife International Ltd. Report to The CSMA Pine Oil Joint Venture. Lab Project ID/Study Number 274A-103.

Guenther E. (1952) The Essential Oils, Volume Six, Individual Essential Oils of the Plant Families, Publishers: Van Nostrand Reinhold Co., Litton Educational Publishing, Inc. New York, NY.

Hagan E. C., Hansen W. H., Fitzhugh 0. G., Jenner P. M., Jones W. I. and Taylor J. M. (1967) Food flavorings and compounds of related structure. II. Subacute and chronic toxicity. *Journal of Food and Cosmetics Toxicology* 5,141-147.

Hall R.L. and Oser, B.L. (1965) Recent progress in the consideration of flavoring ingredients under the food additives amendment. III GRAS Substances. *Food Technology* 19, *151-197.*

Heck J.D., Vollmuth T.A., Cifone M.A., Jagannath D.R., Myhr B., and Curren R.D. (1989) An evaluation of food flavoring ingredients in a genetic toxicity screening battery. *The Toxicologist 9, 257.*

Heil T. P. and Lindsay, R. C. (1990) Environmental and industrial factors relating to flavor tainting of fish in the upper Wisconsin river. *Journal of Environmental Science and Health,* B25(4), 527-552.

Hercules, Inc. (2001) Composition of pine oil blend 1687. Private communication to FFHPVC. Unpublished report.

Heymann E. (1980) Carboxylesterases and amidases. In Enzymatic Basis of Detoxication. Edited by W. B. Jakoby. 2nd ed. p. 291-323. Academic Press, New York.

Horning M.G., Butler C.M. Stafford m., Stillwell R.N., Hill R.M., Zion T.E., Harvey D.J., and Stillwell W.G. (1976) Metabolism of drugs by the epoxide-diol pathway. In: Advances in Mass Spectroscopy in Biochemistry and medicine. Vol. I. p. 91-108. Edited by Frigerio A. and Catagnoli N. Spectrum Publications, New York.

Hoyer H., Keicher, G., and Schubert H. (1959) Farbwerke Hoechst AG, DE 955499 (1956), *Chem Abstr. 53,* 2278f.

Ikawa M., Mosley S., and Barbero B. (1992) Inhibitory effects of terpene alcohols and aldehydes on the growth of green alga Chlorella pyrenoidosa. *Journal of Chemical Ecology* **18**(10), 1755 1760.

Ishidate M., Sofuni T., Yoshikawa K., Hayashi M., Nohmi T., Sawada M., Matsuoda, A. (1984) Primary mutagenicity screening of food additives currently used in Japan. Food and *Chemical Toxicology* **22**(8), *623-636.*

Jenner P.M., Hagan EC., Taylor J.M., Cook E.L., and Fitzhugh O.G. (1964) Food flavourings and compounds of related structure. I. Acute oral toxicity. *Food and Cosmetics Toxicology 2,* 327-343.

Johnson M. C. and Lewis, R.F. (1976) Report to Hercules Inc. on Acute Toxicity Studies with Hercules Fortified Pine-Odor Disinfectant by Industrial Bio-Test Laboratories Inc. Project No. 76-05.

Klimisch H. J., Andreae M., and Tillman U. (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Journal of Regulatory Toxicology and Pharmacology, 25, 1-5.

Larson B., Bonboi-Mingarro, T., Brancaleoni, E., Calogirou, A., Cecinato, A., Coeur, C., Chatzianestis, I., Dusne, M. Frattoni, M., Fugit, J., hanson, U., Jacob, V., Mimkos, N., Hoffman, T.m, Owen, S.,m Perez, R., Reichmann, A., Seufert, G., Staudt, M., Steinbrecher, R. (1997) Sampling and analysis of terpenes in Air. An interlaboratory comparison. *Atomospheric Environment,* **31**(1), 35-49.

Lawrence B. M. (1985) A review of the world production of essential oils *(1984) Perf. and Flav.*, **10**(5), 1-16.

Leinweber F. J. (1987) Possible physiological roles of carboxylic ester hydrolyses. *Drug Metabolism Review 18,* 379-439.

LeLievre M. (1990a) 2,4-Dimethyl 2,7-octadien-4-ol - acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions. Springborn Lab. Inc., Report Submitted to SCM Glidco Organics. SLI Report # 90-g-3476.

LeLievre M. (1990b). 2,4-Dimethyl 2,7-octadien-4-ol - acute toxicity to daphnids (Daphnia pulex) under static conditions. Springborn Lab. Inc., Report submitted to SCM Glidco Organics. SLI Report # 90-g-3494.

Lucas C., Putman, J. M., and Hallagan, J. B. (1999) Flavor and Extract Manufacturers' Association of the United States 1995 Poundage and Technical Effects Update Survey.

Lumdsen A. M. (1998) Plinyl Acetate: Determination of general physico-chemical properties. Safepharm Laboratories, Inc. SPL Project Number: 1044/001.

Mackay D. (1991) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Madyastha K.M. and Srivatsan V. (1988) Biotransformations of alpha-terpineol in the rat: its effects on the liver microsomal cytochrome P-450 system. Bulletin *of Environmental Contamination and Toxicology* 41, 17-25.

Mead C. (1997a) Plinyl Acetate: Algal Inhibition Test. Safepharm Laboratories, Ltd. SPL Project Number 1044/014.

Mead C. (1997b) Plinyl acetate: Assessment of Ready Biodegradability; Manometric Respiratory Test. Safepharm Laboratories, Ltd. SPL Project No. 10441015.

Mead C. (1997c) Plinyl acetate: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge. Safepharm Laboratories. SPL Project No. 1044/018.

Merck & Co., Inc. The Merck Index (1997) 12th Edition, Publishers: Merck Research Laboratories, 12th edition, Whitehouse Station, NJ.

Milas N. A. and Surgenor, D. M. (1946) Decomposition of *t*-butyl hydroperoxide. Journal of *American* Chemical *Society, 68,* 206-201.

Millennium Specialty Chemicals (1999) Glidox 100 (Hydroperoxide Polymerization Initiator) No. 908. Unpublished report.

Millennium Specialty Chemicals (2000) Composition of isolated intermediate 1 N32. Unpublished report.

Misra G., Pavlostathis S.G., Li J., and Perdue E. M. (1995) Characterization and Biodegradation of Selected Monoterpenes. 68th Annual Conference and Exposition, Water Environment Federation; Miami Beach, FL.

Moreno 0. M. (1972a) Linalyl acetate. Acute toxicity studies in rats and rabbits. Unpublished report to RIFM.

Moreno 0. M. (1972b) Acute toxicity studies in rats and rabbits. Unpublished report to RIFM.

Moreno 0. M. (1973) Dihydromyrcenol. Acute toxicity studies in rats and rabbits. Unpublished report to RIFM.

Moreno O.M. (1976) Acute toxicity studies in rats, mice, rabbits and guinea pigs. Unpublished report to RIFM.

Oda Y., Hamono Y., Inoue K., Yamamoto H., Niihara T., and Kunita N. (1978) Mutagenicity of food flavors in bacteria. *Shokuhin Eisei Hen 9. 177-181.*

Palanker A. L. and Lewis C. A. (1979) Acute toxicity studies in rats and rabbits. Unpublished report to RIFM.

Parent R.A. (1988) Teratogenicity study in albino rats with pine oil blend. A report submitted by Pine Oil Joint Venture to Chemical Specialties Manufacturers Association.

Parke D. V., Rahman K.H. M.Q., and Walker R. (1974a) The absorption, distribution and excretion of linalool in the rat. *Biochem. Soc. Trans. 2, 612-615.*

Parke D. V., Rahman K.H. M.Q., and Walker R. (1974b) Effect of linalool on hepatic drug-metabolizing enzymes in the rat. *Biochem. Soc. Trans. 2 61.5618.*

Phillips, J. C. Kingsnorth, J., Gangolli, S. D. and Gaunt, I. F. (1976) Studies on the absorption, distribution and excretion of citral in the rat and the mouse. *Food and Chemical Toxicology 14, 537-540.*

Proctor & Gamble Co. (1996) Submission to EPA. Unpublished report.

PSMA (Pine Source Materials Association) (2000) Private communication to FFHPVC.

Putman D. L. (1987) Micronucleus Cytogenetic Assay in Mice. Pine oil Blend. Microbiological Associates, Inc. Report to Chemical Specialties Manufacturers Association. Lab. Study Number T5366.122.

Quest International Ltd. (1991) Biodegradability study of linalyl acetate in a sealed vessel test, Private communication to RIFM.

Quest International Ltd. (1994a) Biodegradability test of terpinyl acetate in a sealed vessel test. Private communication to RIFM.

Quest International Ltd. (1994b) The ultimate biodegradability of tetrahydrolinalool in the sealed vessel test. Private communication to RIFM.

Quest International Ltd. (1994c) Biodegradability study of dimyrcetol in a sealed vessel test. Private communication to RIFM.

Quest International Ltd. (1994d) Biodegradability test of linalool in a sealed vessel test. Unpublished report.

Rahman K. A. Q. M. Q. (1974) Ph.D. thesis, University of Surrey. Cited in Chadha A. and Madyastha K. M. (1984) Metabolism of geraniol and linalool in the rat and effects on liver and lung microsomal enzymes. *Xenobiotica 14, 365-374.*

Robbins G. R. (1994) Acute inhalation toxicity study in rats. Pine Oil. Cosmopolitan Safety Evaluation, Inc. Report to CSMA Pine Oil Joint Venture. Lab Project ID/Study Number *c3371.*

Rockwell P. and Raw I. (1979) A mutagenic screening of various herbs, spices, and food additives. *Nutr. Cancer* 1(4), IO-15

Rudio J. (1991) Test Report Nr 90-57/B. Linalool Synthetic. Biodegradation unpublished report.

Ryckman, Edgerley, Burbank and Associates, Inc. (1966) Unpublished report to Hercules Powder Company.

Ryckman, Edgerley, Burbank and Associates, Inc. (1965) Investigation of treatability and fish toxicity of certain manufacturing products and wastes and their decay under stream conditions. Unpublished report to Hercules Powder Company.

Sanders A. (1997). Plinyl Acetate: Acute Oral Toxicity (Limit Test) in the Rats. Safepharm Laboratories Ltd. SPL Project No. 1044/004.

Schmidt G. S. and Fisher G. S. (1959) The decomposition of pinane hydroperoxide. *Journal of American Chemical Society, 81,* 445-448.

Stofberg J. and Grundschober, F. (1987) The consumption ratio and Food Predominance of flavoring materials. *Perfumer & Flavorist 12, 27-56.*

Stofberg J. and Kirschman, J. C. (1985) The consumption ratio of flavoring materials: A mechanism for setting priorities for safety evaluation. *Food and Chemical Toxicology 23,* 857-860.

Stroh J., Wan, M. T., Isman, M. B. and Moul, D. J. (1998) Evaluation of the acute toxicity to juvenile pacific coho salmon and rainbow trout of some plant essential oils, a formulated product, and the carrier. *Bull. Enviro. Confam. Toxicol. 60, 923-930.*

Syracuse Research Corporation (SRC) Private communication to FMA.

Thomas 0. N. (1997) Plinyl Acetate: Twenty-Eight day Repeated Dose Oral (Gavage) Toxicity Study in the Rat. Safepharm Laboratories, Ltd. SPL Project No. 10441009.

Thompson P. W. (1997) Plinyl Acetate: Reverse Mutation Assay "Ames Test" Using Salmonella Typhimurium. Safepharm Laboratories Ltd. SPL Project Number 1044/010.

Toxic Substances Control Act (TSCA) PL 94469 (1978) U. S. Environmental Protection Agency, Candidate list of chemical substances, Addendum III, Chemical Substances of Unknown or Variable Composition, Complex reaction products and biological materials.

TOXIKON, Inc. (1990) Analysis of Pine oil Blend, CMSA Compositie Sample 110990. Private Communication to FFHPVC. Unpublished report.

Tremain S. P. (1997). Plinyl Acetate: Determination of Vapor Pressure. Safepharm Laboratories Ltd. SPL Project No. 1044/003.

Trubeck Laboratories, Inc. (1958) Toxicologic1 screening of linalyl acetate, linalyl isobutyrate and geranyl acetate in rats. Class VIII. Unpublished report.

UCC Business Confidential (1986). Ecological Fate and Effects Testing of UCC Products and Wastewaters During 1985.

Van Duuren B., Blazej T., Goldschmidt B. Katz C., Melchionne S., and Sivak A. (1971) Cocarcinogenesis studies on mouse skin and inhibition of tumor induction. *Journal of the National Cancer Institute* **45**(5), *1039-I 044.*

Ventura P., Schiavi M., Serafini S., and Selva S. (1985) Further studies of trans-sobrerol metabolism: rat, dog, and human urine. *Xenobiofica* ***15,*** *317-325.*

Villa M. E. (1994) Analysis of pine-sol dilutable No. 012494-I. The Chlorox Company Project No. 18424. Private communication to FFHPVC. Unpublished report.

Vollmuth T.A., Bennett, M.B., Hoberman, A.M. and Christian, M.S. (1995) An evaluation of food flavoring ingredients using an in vivo reproductive and developmental toxicity screening test. *Teratology* **41**(5), *597.*

Vuilleumeier C., Flament I. and P. Sauvegrain (1995) Headspace analysis study of evaporation rate of perfume ingredients applied onto skin. *International Journal of Cosmetic* Science **17**(1), 61-76.

Webb R. L. (1960) The Glidden Company US *2902510 (1959). Chem. Absfr. 54,* 1292d.

Webb R. L. (1964) The Glidden Company US *3076839 (1958) Chem. Absfr. 60,* 3020a.

Wetton P. M. (1997a). Plinyl Acetate: Acute Toxicity to Rainbow Trout (Oncohychus mykiss). Safepharm Laboratories Ltd. SPL Project Number: 1044/012.

Wetton P. M. (1997b) Plinyl Acetate: Acute Toxicity to Daphnia Magna. Safepharm Laboratories, Inc. SPL Project Number: 1044/013.

Williams R.T. (1959) Detoxication Mechanisms. The Metabolism and Detoxication of Drugs, Toxic Substances, and Other Organic Compounds, 2nd ed., Chapman and Hall, Ltd., London, p. 119-120.

Wright N. P. (1997) Plinyl Acetate: chromosome aberration test in human lymphocytes in vitro. Safepharm Laboratories Ltd. SPL Project No. 1044/011.

Yamaguchi T., Caldwell J., and Farmer P. B. (1994) Metabolic fate of [3H]-l-menthol in the rat. *Drug Metabolism and Disposition 22, 616-624.*

Yamahara J., Kimura H., Kobayashi M., Okamoto T., Sawada T., Fujimura H., and Chisaka T. (1985) Cholagogic action and characteristics of (+/-)*alpha*-terpineol-beta-D-O-glucopyranoside, a new monoterpenoid glucoside. *Chemical and Pharmaceutical Bulletin* 33, 1669-1675.

Yang L. L. (1987) CHO/HGPRT Mutation Assay. Pine Oil Blend. Microbiological Associates Inc Report to Chemical Specialties Manufacturers Association. Lab. Study No. T5366.332001.

Yoo Y.S. (1986) Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. *Journal of the Osaka City Medical* Center 34, 267-288.